



TITLE:

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Karyotypes of Three Rat Species (Mammalia: Rodentia: Muridae) from Hainan Island, China, and the Valid Specific Status of *Niviventer lotipes*

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The karyotypes of three rat species from Hainan Island, China, were examined. *Niviventer fulvescens* (Gray, 1847) had $2n=46$ and $FN=64$, similar to the karyotypes reported for *N. fulvescens* from Southeast Asia, while *Niviventer lotipes* (Allen, 1926) had $2n=52$ and $FN=66$, which is distinct from the known karyotypes of other *Niviventer* species. *Niviventer lotipes* was recently considered conspecific with *N. tenaster* (Thomas, 1916), but the two were found to have extremely different karyotypes ($2n=52$ and $FN=66$ in *N. lotipes*; $2n=46$ and $FN=54$ in *N. tenaster*). Therefore, in this paper *N. lotipes* is considered a valid species for the first time; it is distinct from *N. tenaster* and endemic to Hainan Island, where *N. lotipes* is differentiated from *N. fulvescens* by larger body and skull sizes, a shorter tail, and darker coloration. *Rattus nitidus* (Hodgson, 1845) from Hainan Island had $2n=42$ and $FN=62$, which is similar to the reported karyotypes of conspecific populations in Southeast Asia.

Key words: karyotype, *Niviventer fulvescens*, *N. lotipes*, *Rattus nitidus*, taxonomy

INTRODUCTION

Hainan Island is a continental island in the South China Sea ($18^{\circ}09'–20^{\circ}10'N$, $118^{\circ}03'–111^{\circ}03'E$); it has an area of 33,920 km², coastal length of 1528 km, and a maximum elevation of 1867 m at the top of Mt. Wuzhi (Jiang et al., 2002). Several mammal surveys have been conducted on Hainan Island (Allen, 1906, 1909; Allen, 1940; Shaw et al., 1966; Guangdongsheng Kunchong Yanjiusuo Dongwushi and Zhongshan Daxue Shengwuxi, 1983), and about 76 mammal species are known from the island (Guangdongsheng Kunchong Yanjiusuo Dongwushi and Zhongshan Daxue Shengwuxi, 1983). The mammal fauna on Hainan Island is closely related to those in southern China and Vietnam.

The taxonomy of *Niviventer* species on Hainan Island is unclear and controversial. Several studies have recognized two species from Hainan Island, using different combinations of names and causing a great deal of taxonomic confusion, while many other studies have recognized a single species (Corbet and Hill, 1992; Musser and Carleton, 1993; Wang, 2002). According to a recent checklist by Musser and Carleton (2005; also see Musser et al., 2005),

two *Niviventer* species occur on Hainan Island, *N. fulvescens* (Gray, 1847) and *N. tenaster* (Thomas, 1916). The former is widely distributed, from the southern Himalayas through Bangladesh and South China (including Hainan Island and Hong Kong), and from Indochina to the Sunda Shelf on peninsular Thailand, the Malay Peninsula, Sumatra, Java, and Bali. The latter is distributed in the mountains of west-central and southern Myanmar, Northwest Thailand, southern Cambodia, southern Laos, Vietnam, the Tengchong region of western Yunnan, and Hainan Island. *Niviventer tenaster* was first described by Thomas (1916) from southern Burma and included two junior synonyms, *N. champa* (Robinson and Kloss, 1922) from southern Vietnam and *N. lotipes* (Allen, 1926) from Hainan Island.

We conducted a small-mammal survey on Hainan Island in February 2005 and collected three rat species identified using morphological features as *N. fulvescens*, *N. lotipes* (Allen, 1926), and *Rattus nitidus* (Hodgson, 1845). Morphological and karyological examinations were conducted for taxonomic purposes, and this is the first karyotype report for Hainan samples of these species. Karyological data reported in this paper suggest that *N. lotipes* is a valid species distinct from *N. tenaster*, as discussed below; thus, we use the specific name *N. lotipes* for Hainan samples, rather than *N. tenaster*.

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MATERIALS AND METHODS

In February 2005, we trapped samples of small mammals on Mt. Diaoluo (18°44.7'N, 109°53.2'E; 900 m above sea level) and Tianchi, Jianfengling (18°44.5'N, 108°51.6'E; 860 m a.s.l.) on Hainan Island, China. We collected 13 individuals encompassing three species. Voucher specimens prepared as flat skin and skull specimens are deposited at the Hainan Wildlife Conservation and Research Center, Department of Biology, Hainan Normal University, but have not yet been cataloged. The specimens examined in the study include the following; numbers are field numbers given by MM.

Rattus nitidus (n=1). Tianchi, Jianfengling, M4521 (female).

Niviventer fulvescens (n=11). Mt Diaoluo (n=3), M4504 (male), M4511 (female), M4512 (male). Tianchi, Jianfengling (n=8): M4519 (female), M4520 (female), M4522 (male), M4523 (male), M4528 (male), M4529 (male), M4531 (male), M4532 (male).

Niviventer lotipes (n=1). Tianchi, Jianfengling: M4530 (male).

We weighed each specimen and took external measurements (total length, tail length, ear length, and hindfoot length with and without claws) using standard methods. Eighteen measurements were taken on the skull following Motokawa et al. (2003, 2004). All specimens had moderately worn molars.

Species identification was made based on morphology in external and cranial features following Allen (1926), Guangdongsheng Kunchong Yanjiusuo Dongwushi and Zhongshan Daxue Shengwuxi (1983), and Lunde and Son (2001). Although the taxonomy of *Niviventer* species is controversial, we could identify two *Niviventer* species on Hainan Island with clear morphological differences referable to *N. lotipes* and *N. fulvescens*. *Niviventer lotipes* has larger body size, larger ears, dorsal coloration of mixed ochraceous and black, ventral coloration of sulphury white, and a deeper braincase; while *N. fulvescens* has smaller body size, smaller ears, dorsal coloration of rusty brown with reddish tone, ventral coloration of white, and a shallower braincase (Allen, 1926; Guangdongsheng Kunchong Yanjiusuo Dongwushi and Zhongshan Daxue Shengwuxi, 1983).

Karyological analyses were made from tail or lung tissue-culture cells following the methods described in Harada and Yosida (1978). The C-band technique was applied for differential staining following Sumner (1972) to identify sex chromosomes; the Y chromosome was entirely stained. Chromosomal terminology and abbreviations follow Levan et al. (1964): M, metacentric; SM, submetacentric; ST, subtelocentric; and A, acrocentric. Chromosome number (2n) and fundamental number (FN, including two X chromosomes) were calculated.

RESULTS

External features and skulls are shown in Figs. 1 and 2, respectively. External and cranial measurements are given in Table 1. *Niviventer lotipes* differed from *N. fulvescens* in having darker coloration and a larger body size. *Niviventer lotipes* had a shorter tail (tail length was 106.5% that of the head and body length) than *N. fulvescens* (107.0–137.4%). In addition, *N. lotipes* was larger than the maximum values for *N. fulvescens* in many external and skull measurements (Table 1). In addition to the morphological differences used for species identification between the two *Niviventer* species, as described in the Materials and Methods section, *N. lotipes* differs from *N. fulvescens* in having a wider, deeper rostrum, a more expanded zygomatic arch, a longer auditory bulla, and better-developed molars (Fig. 2).

Karyotypes of the three rat species are shown in Fig. 3. The autosomes of *N. fulvescens* consisted of three small metacentric pairs (Fig. 3A, 1–3), six subtelocentric pairs

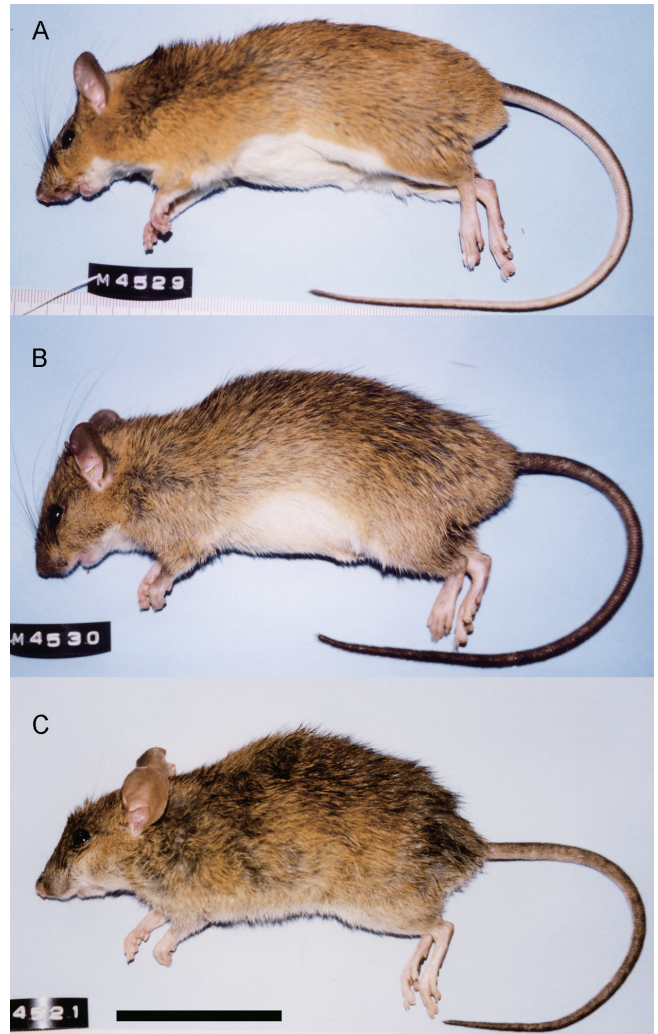


Fig. 1. The external features of (A) *Niviventer fulvescens* (M4529), (B) *N. lotipes* (M4530), and (C) *Rattus nitidus* (M4521) collected from Hainan Island, China. Scale bar=50 mm.

gradually decreasing in size (4–9), and 13 acrocentric pairs gradually decreasing in size (10–22). The X and Y chromosomes were large acrocentric and small acrocentric, respectively. The 2n and FN values were calculated to be 46 and 64, respectively.

The autosomes of *N. lotipes* consisted of two small metacentric pairs (Fig. 3B, 1 and 2), one large submetacentric pair (3), four large- to medium-sized subtelocentric pairs (4–7), and 18 acrocentric pairs (8–25). The X and Y chromosomes were medium-sized acrocentric and small submetacentric, respectively, and the 2n and FN were calculated to be 52 and 66, respectively.

The karyotype of the one female *R. nitidus* consisted of eight medium to small metacentric pairs (Fig. 3C, 1–8), one large and one small subtelocentric pair (9, 10), and 11 large to small acrocentric pairs (11–21). Based on comparisons with published data on conspecific populations, one acrocentric pair is thought to be the X chromosome. If so, the autosomes consisted of eight metacentric pairs, two subtelocentric pairs, and ten acrocentric pairs. The 2n and FN values were calculated to be 42 and 62, respectively.

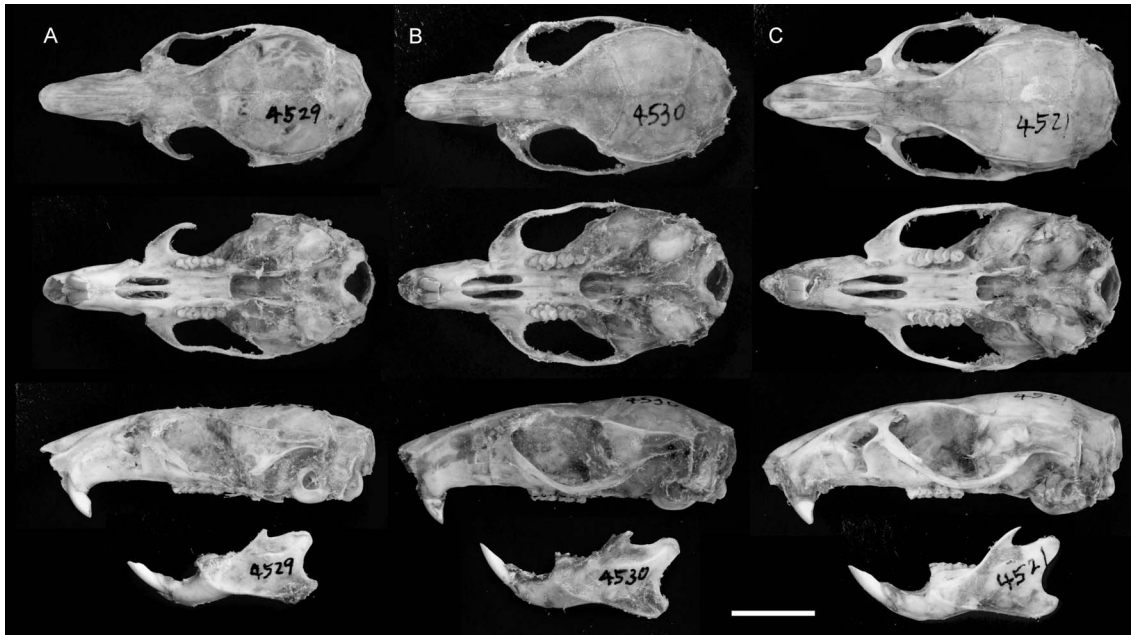


Fig. 2. The skulls of (A) *Niviventer fulvescens* (M4529), (B) *N. lotipes* (M4530), and (C) *Rattus nitidus* (M4521) collected from Hainan Island, China. Scale bar=5 mm.

Table 1. External and cranial measurements (in mm except for BW in gram) of *Niviventer fulvescens*, *N. lotipes*, and *Rattus nitidus* from Hainan Island, China. Values for *N. lotipes* with asterisks are larger than the maximum values for *N. fulvescens*.

Measurements	<i>N. fulvescens</i> 8 males, 3 females			<i>N. lotipes</i> 1 male	<i>R. nitidus</i> 1 female
	Mean±SD	Range	N		
Body weight (BW)	56.3 ±15.2	(38.6 – 86.0)	11	97.0*	77.0
Head and body length (HB, =TL–T)	135.3 ±12.0	(123.0 –159.0)	10	170.0*	151.0
Total length (TL)	292.4 ±31.0	(259.0 –350.0)	10	351.0*	311.0
Tail length (T)	161.9 ±19.8	(137.0 –193.0)	9	181.0	160.0
Tail ratio (TR, % of T to HB)	120.6 ± 9.0	(107.0 –137.4)	9	106.5	106.0
Ear length	19.1 ± 1.3	(17.0 – 21.1)	11	22.1*	22.1
Hindfoot length without claw	27.9 ± 1.0	(6.4 – 29.9)	11	28.3	31.1
Hindfoot length with claw	29.1 ± 1.1	(27.7 – 31.2)	11	30.0	33.1
Occipitonasal length	34.28± 2.20	(31.94– 38.72)	11	38.33	40.37
Condylobasal length	30.52± 2.01	(28.37– 33.93)	11	34.81*	37.34
Greatest zygomatic breadth	15.50± 0.51	(14.85– 16.43)	10	17.93*	18.93
Breadth of the braincase	14.84± 0.44	(14.13– 15.59)	11	15.42	15.85
Breadth across the occipital condyles	7.74± 0.32	(7.22– 8.15)	11	7.96	7.75
Least interorbital breadth	5.94± 0.21	(5.60– 6.24)	11	6.56*	5.73
Length of the nasals	12.55± 1.07	(10.81– 14.03)	11	16.37*	17.12
Breadth of the rostrum	5.48± 0.36	(5.10– 6.26)	11	5.70	6.78
Postpalatal length	11.69± 1.11	(10.38– 13.39)	11	14.45*	13.70
Length of the bony palate	6.40± 0.35	(5.80– 7.18)	11	6.64	7.96
Length of the diastema	8.47± 0.80	(7.62– 10.08)	11	10.35*	11.30
Length of the incisive foramen	5.52± 0.49	(4.88– 6.40)	11	6.76*	7.74
Breadth across the 1st upper molars	6.92± 0.21	(6.69– 7.25)	11	7.59*	8.65
Breadth of the zygomatic plate	3.17± 0.25	(2.82– 3.62)	11	4.41*	4.36
Length of the auditory bulla	4.97± 0.44	(4.59– 6.00)	11	5.95	5.88
Alveolar length of the maxillary toothrow	5.78± 0.15	(5.57– 6.05)	11	6.96*	6.47
Coronal width of the 1st upper molar	1.66± 0.04	(1.62– 1.73)	11	1.90*	1.87
Height of the braincase	9.98± 0.41	(9.26– 10.77)	11	10.83*	11.50

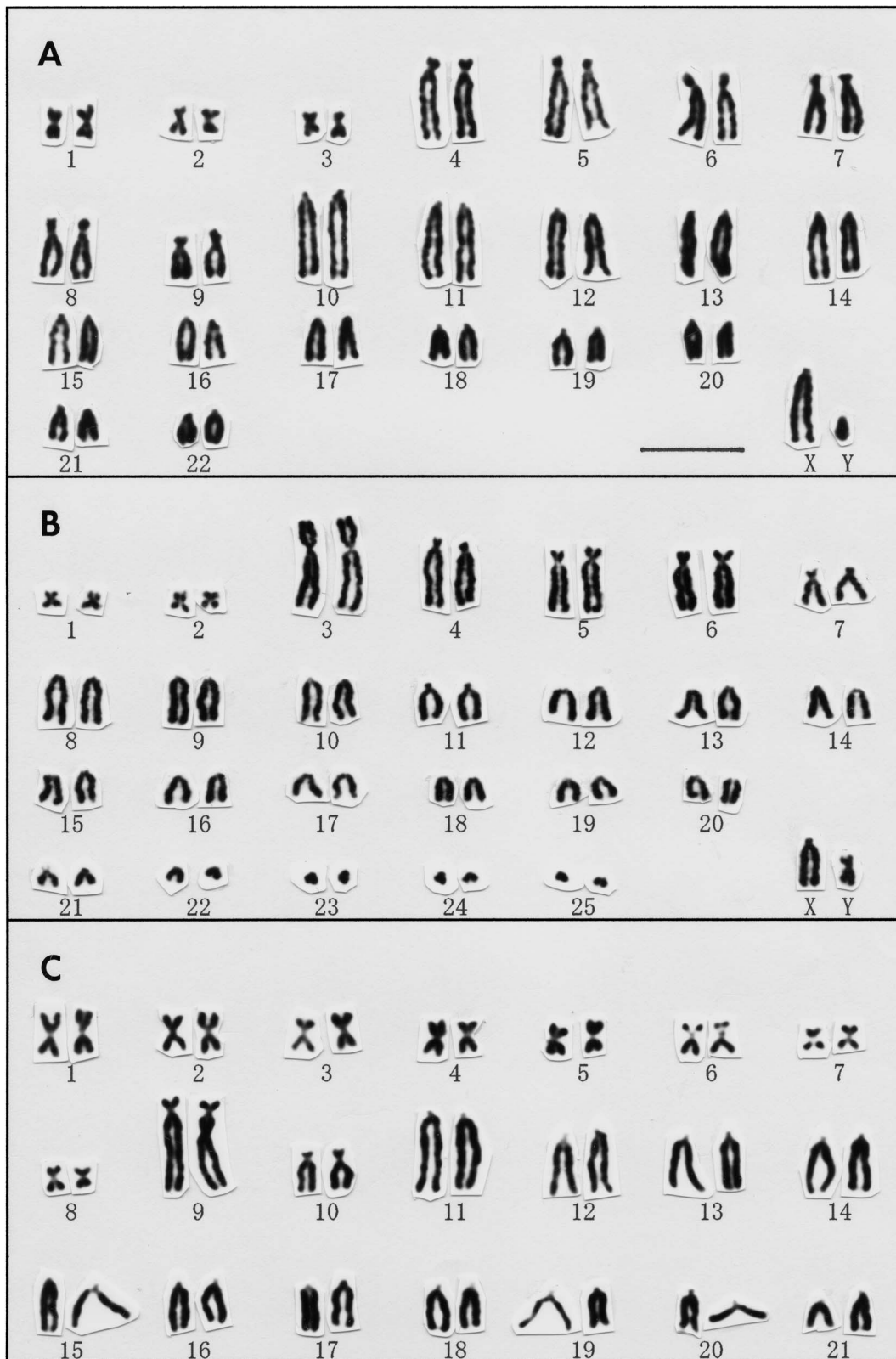


Fig. 3. Conventional karyotypes of (A) *Niviventer fulvescens* (M4529), (B) *N. lotipes* (M4530), and (C) *Rattus nitidus* (M4521) collected from Hainan Island, China. Scale bar=10 μ m.

DISCUSSION

Rattus nitidus is indigenous to mainland Southeast Asia and occurs in southern China (including Hainan Island), Vietnam, Laos, northern Thailand, Burma, Bangladesh, Nepal, Bhutan, and northern India; it is also found on the islands of central Sulawesi, Luzon Island in the Philippines, Pulau Seram in the Moluccas, the Vogelkop Peninsula of the Province of Papua, and the Palau Islands, probably due to human-mediated introductions (Aplin et al., 2003; Musser and Carleton, 2005). The karyotype of *R. nitidus* from Hainan Island (2n=42, FN=62) is not very different from previous reports for the same species from Kathmandu, Vietnam, and Thailand (2n=42, FN=58–62) based on the available figures (Markvong et al., 1973a, b; Cao and Tran, 1984; Duncan and Van Peenen, 1971; Gadi and Sharma, 1983). Differences in the 2n and FN estimates may be caused by different preparation methods or by interpretive differences among studies.

Musser and Carleton (2005) recognized 17 species within *Niviventer*, a genus that is very taxonomically confused. In particular, the boundaries between *N. confucianus*, *N. fulvescens*, *N. tenaster*, and *N. niviventer* remain very uncertain in locations where two species overlap, which may be attributable to poorly defined species limits and extensive geographic variation within each species (Osgood, 1932; Ellerman, 1961; Abe, 1983; Corbet and Hill, 1992; Lunde and Son, 2001; Musser and Carleton, 2005). To clarify these issues, interspecific and geographic variation have been studied in several localities (Niethammer and Martens, 1975; Abe, 1977, 1983; Lunde and Son, 2001; Lunde et al., 2003; Musser et al., 2005). In Indochina, *N. fulvescens* and *N. confucianus* were reported under several species names, such as *N. fulvescens*, *N. confucianus*, *N. niviventer*, *N. rapit*, and *N. bukit*. Because the genus *Niviventer* was described as *Rattus* by Marshall (1976), these rats were previously placed in the genus *Rattus* and referred to as such even after Marshall (1976). Due to past confusion, many previous reports on the distributions and karyotypes of these species are difficult to interpret without examining the voucher specimens.

The occurrence of two species of *Niviventer* on Hainan

Island was widely known (Allen, 1940; Ellerman, 1941; Ellerman and Morrison-Scott, 1951; Shaw et al., 1966; Guangdongsheng Kunchong Yanjiusuo Dongwushi and Zhongshan Daxue Shengwuxi, 1983; Huang et al., 1995; Zhang, 1997; Musser and Carleton, 2005) since Allen's (1926) report, although several studies recognized only one species (Corbet and Hill, 1992; Musser and Carleton, 1993; Wang, 2002). Allen (1926) reported *N. fulvescens* as *Rattus huang*, which was originally described by Bonhote (1905, 1906) from Kuatung, Fokien (=Fujian), China. In addition, Allen (1926) reported *N. lotipes* as a new subspecies on Hainan Island but called it *Rattus confucianus lotipes*. Thereafter, these two species were often confused, with the general taxonomic confusion of these rats extending to Indochina. Our study indicates that *N. lotipes* is larger than *N. fulvescens*. Although these two species are sympatric, at least at Tianchi, they are distinguishable and can be identified by color pattern, the body to tail length relationship, and some skull measurements.

Musser and Carleton (2005) and Musser et al. (2005) revised the systematics and distribution of the genus *Niviventer* and clarified the systematics and distribution of the four Indochinese species *N. confucianus*, *N. fulvescens*, *N. tenaster*, and *N. langbianis* (also see Musser, 1973, 1981). Musser and Carleton (2005) and Musser et al. (2005) recognized the Hainan species to be *N. fulvescens* and *N. tenaster*. Their studies were the first to consider *lotipes* as a junior synonym of *N. tenaster*. However, as shown here, the karyotype of *N. lotipes* is clearly distinct from that of *N. tenaster*.

The available karyotype information on *N. confucianus*, *N. fulvescens*, *N. tenaster*, and *N. langbianis* is summarized in Table 2 (Yong, 1969b; Yosida, 1973; Duncan et al., 1974; Cao and Tran, 1984; Jiang, 1995; Wang et al., 1997, 2003; Baskevich and Kuznetsov, 2000). As noted above, in several reports, determining whether the species studied was *N. fulvescens* or *N. confucianus* (or another) is difficult; ambiguous reports are not included in Table 2 (2n=46, FN=54–62; Yong, 1969a; Duncan et al., 1970; Yosida, 1973; Markvong et al., 1973a, b; Tsuchiya et al., 1979; Cao and Tran, 1984; Jiang, 1995; Baskevich and Kuznetsov, 2000). The Hainan *N. fulvescens* karyotype (2n=46, FN=64)

Table 2. Reported karyotypes for *Niviventer lotipes*, *N. fulvescens*, *N. confucianus*, *N. tenaster*, and *N. langbianis*. Reports with ambiguous identifications were excluded (see text).

Species	Locality	2n	FN	M	SM	ST	A	X	Y	Reference
<i>N. lotipes</i>	Hainan Island	52	66	2	1	4	18	A	SM	Present study
<i>N. fulvescens</i>	Hainan Island	46	64	3	0	6	13	A	A	Present study
	Guangdong	46	64	3	0	5	14	ST	A	Jiang (1995)
	Hong Kong (as <i>R. huang</i>)	46	62	3	0	4	15	ST	A	Yong (1969b)
	Hong Kong (as <i>R. huang</i>)	46	60	3	0	4	15	A	A	Yosida (1973)
	Java (as <i>R. niviventer temmincki</i>)	46	60	3	0	4	15	A	A	Duncan et al. (1974)
	Java (as <i>R. niviventer treubii</i>)	46	60	3	0	4	15	A	A	Duncan et al. (1974)
<i>N. confucianus</i>	Guangdong	46	54	3	0	0	19	SM	A	Jiang (1995)
	Shandong	46	60	4	0	2	16	SM	A	Wang et al. (1997, 2003)
	Shaanxi	46	62	4	1	1	16	A	A	Wang et al. (2003)
<i>N. tenaster</i>	Vietnam	46	54	3	0	0	19	SM	A	Baskevich and Kuznetsov (2000)
<i>N. langbianis</i>	Vietnam (as <i>R. cremoriventer</i>)	46	54	3	0	1	18	A	A	Cao and Tran (1984)
	Vietnam (female only)	46	54	3	0	1	18	A?	?	Baskevich and Kuznetsov (2000)

is not very different from the previous reports on conspecific populations or from reported karyotype figures. One difference is the number of subtelocentric autosome pairs: six versus four or five from other reports.

Variation in the number of subtelocentric pairs (and differences in the acrocentric pairs) in *N. fulvescens* and *N. confucianus* may have not been due to the preparation methods or differences in interpretation among studies, because in published karyotype figures, the subtelocentric pairs have easily observed short arms. The number of subtelocentric pairs also differs between *N. fulvescens* and *N. confucianus* and is not continuous: one or two in *N. confucianus* versus four to six in *N. fulvescens*. These karyological characteristics may be important characters separating two (*N. fulvescens* and *N. confucianus*) or more species, and defining intraspecific geographic variation. Future karyological studies, as well as morphological re-identification of specimens used in previous karyological studies, are necessary to correctly evaluate chromosome variation in the genus *Niviventer*.

The karyotype of *N. lotipes* is quite different from that of the other *Niviventer* species in having larger 2n and FN values (Table 2; *N. cremoriventer*, 2n=46, FN=54, Yong, 1969a; 2n=46, FN=60, Duncan et al., 1974; *N. cameroni*, 2n=46, FN=60, Yong, 1969a; *N. hinpoon*, 2n=46, FN=54, Marshall, 1977; *N. lepturus*, 2n=46, FN=52, Duncan et al., 1974; *N. culturatus*, 2n=46, FN=52, Yu et al., 1996; *N. coninga*, 2n=46, FN=54, Yu et al., 1996). Although 2n is always 46 in other *Niviventer* species, *N. lotipes* has 52; FN ranges between 52 and 64 in other species, but is 66 in *N. lotipes*. Three small metacentric pairs are characteristic of the genus *Niviventer*, but *N. lotipes* has only two small metacentric pairs. The karyotype of *N. tenaster* from the Dalat Plateau in southern Vietnam (Baskevich and Kuznetsov, 2000) was 2n=46 and FN=54, similar to karyotypes of the other *Niviventer* species, but much different from the karyotype of *N. lotipes*. Values of 2n and FN similar to those of *N. lotipes* are found in the rat genus *Maxomys* in Indochina, according to the karyotype checklist by Rickart and Musser (1993), but the karyomorphs of *N. lotipes* and *Maxomys* species are quite different: *M. moi* from Vietnam, 2n=52, FN=74 (Duncan and Van Peenen, 1971) and *M. surifer* from Vietnam, Thailand, and the Malay Peninsula, 2n=52, FN=66 (Duncan and Van Peenen, 1971; Markvong et al., 1973a, b; Yong, 1969a). These karyological differences suggest that the Hainan *N. lotipes* represents a valid species, recognized for the first time in this paper. However, the morphological characters are not very different between *N. lotipes* and *N. tenaster* based on a comparison of skull pictures of our specimen with figures given in Musser (1973) and Musser et al. (2005). Musser and Carleton (2005) suggested morphometrical similarity between *N. tenaster* and *N. lotipes* based on unpublished data.

Jiang (1995) reported a rat karyotype from Hainan Island, *R. confucianus lotipes*, as 2n=46 and FN=54, which is similar to the *N. confucianus* karyotype from Guangdong Province. However, Jiang (1995) did not provide any information about species identification, and thus the species this karyotype represents is uncertain. As noted above, the identification of rats from China and Southeast Asia is often confused, and thus reexaminations of voucher specimens

are required to confirm the species identification of rats with a 2n=46 and FN=54 karyotype from Hainan Island.

Finally, we provide a taxonomic history of the two *Niviventer* species on Hainan Island. *Niviventer lotipes* (Allen, 1926), determined to be a valid species in this paper, has been treated as *Rattus confucianus lotipes* by Allen (1926, 1940) and Ellerman (1941); *R. niviventer lotipes* by Ellerman (1949), Ellerman and Morrison-Scott (1951), Shaw et al. (1966), and Guangdongsheng Kunchong Yanjiusuo Dongwushi and Zhongshan Daxue Shengwuxi (1983); *N. confucianus* by Corbet and Hill (1992); *N. fulvescens* by Musser and Carleton (1993); *N. confucianus lotipes* by Huang et al. (1995), Deng et al. (2000), and Wang (2002); *R. niviventer* by Zhang (1997); and *N. tenaster* by Musser and Carleton (2005). *Niviventer fulvescens* (Bonhote, 1906) has been treated as: *R. fulvescens huang* by Allen (1940), Shaw et al. (1966), and Guangdongsheng Kunchong Yanjiusuo Dongwushi and Zhongshan Daxue Shengwuxi (1983); *R. huang* by Allen (1926), Ellerman (1941), and Ellerman and Morrison-Scott (1951); *N. confucianus* by Corbet and Hill (1992); *N. fulvescens* by Musser and Carleton (1993); *N. fulvescens huang* by Huang et al. (1995); *R. fulvescens* by Zhang (1997); *N. confucianus lotipes* by Wang (2002); and *N. fulvescens* by Musser and Carleton (2005).

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